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# THE CONDITIONS FOR CONJUGATION IN DIVERSE RACES OF PARAMOECIUM

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# The Conditions for Conjugation in Diverse Races of Paramoecium.

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# INTRODUCTION.

Maupas ('89) was one of the first to suggest differences between nearly related species of Infusoria in respect to their tendency to conjugate. He found for example, that Colpidium colpoda would conjugate more readily than the similar species C.truncatum. Like differences have been shown by later investigators to obtain in other genera,as in the two species of Colpoda studied by Enriques ('08). It had long been known,too,that cultures of the same species differ much as regards susceptibility. Most authors attributed these differences to environmental factors,as mixed cultures of uncertain derivation were used in most cases. As pure,or "pedigreed",races began to be used htese cultural diversities were conceived of as having possibly a racial basis. If diversities with respect to size,shape and division rate have their origin in heredity,may it not be that differences in the ability to conjugate are based upon hereditary factors?

This view has led to some controversy in recent years in regard to the existence of so-called "non-conjugating races",calling into question the universality of fertilization amongst the Infusoria. That the process may be postponed indefinitely in Paramoecium aurelia,provided the cultural conditions are kept uniform and favorable,seems probable in the light of Woodruff's extended work,for he maintained one strain thru more than six thousand generations without conjugation (1917). This same race of organisms was subjected to conditions which favor conjugation

with positive results (Woodruff,'14). It would appear from these results that,in Par.aurelia at least,the occurrence of conjugation is facultative under uniform conditions of environment. Other reports,moreover,would indicate that conjugation is possibly lacking in the life-history of some races of Paramoecium,or recurrent only after very long time intervals in others. Thus Jennings ('10) maintains that there exist in these organisms striking racial differences with respect to conjugation. In some of the strains employed in his work normal"epidemics" occurred frequently,sometimes apparently under uniform conditions of existence,in some only infrequently,and in others not at all. He emphasized also growth and nutrition as important factors in regulating conjugation. Thus a period of rapid multiplication followed by a decline in the division rate usually gave an epidemic of conjugation in the majority of cases. A preliminary period of semi-starvation (reduction in division rate) of two weeks or longer made the organisms more susceptible when the above mentioned procedure was followed. Some few cultures (races) failed to yield conjugants even when the most favorable conditions were afforded. Two species of Paramoecium (caudatum and aurelia) were used in his work--and the same kind of differences were found in each:there were races which conjugated freely,often at frequent intervals,others which did not;altho P.caudatum showed less frequent epidemics and was characterized by a greater number of the so called "non-conjugating " races.

The process of nuclear reorganization,or endomixis,

discovered by Hertwig ('89),and worked out by Woodruff and Erdmann ('14 and '16),would seem to suffice in maintaining the normal vegetative processes in Paramoecium in the entire absence of conjugation. Even in such a form as the hypotrichate Uroleptus,according to the work of Calkins ('19,two papers),conjugation is probably not essential to the continued life of the race. For,altho a nuclear reorganization process occurs only within the cyst,animals can be derived from the cysts which develop with the same renewed vigor as characterizes exconjugants.

The direct effect of altered environmental conditions in determining conjugation was more strongly emphasized by Enriques ('09) who employed solutions of electrlytes to induce epidemics of conjugation in Cryptochilum nigricans. His studies led him to believe that the process is in no way bound up with an inferred "life-cycle",and so is not dependent primarily upon internal factors. This same general method was adopted later by Zweibaum ('12) in a careful study of a race of Paramoecium caudatum. During the early history of this race conjugation could be induced to occur at times simply by subjecting samples of the flourishing cultures to the action of certain salt solutions. Later it was foumd necessary to subject the cultures to a preliminary period of partial starvation ("disette") lasting from 5 to 6 weeks. Cultures which had been maintained under conditions of steady growth ("cultures continuative") no longer gave conjugants when treated with salts,except in rare instances,and then in very small numbers. From the "dormant" cultures,however,conjugants were readily obtained after allowing the organisms to multiply rapidly in a rich culture medium,changed every

three or four days during a period of about one week, then subjecting them to the action of the salt solutions in optimal concentrations (as determined previously by experiment). This last process is to be carried out with large numbers of Paramoecia, in a small culture dish, the organisms being thus limited to a small amount of nutriment. The solutions of salts are then added in the ratio of three parts of salt solution to one part of culture (15 cc.:5 cc.). The essential steps in the procedure are seen to be those laid down by Maupas--with an added factor, namely, the treatment with a chemical reagent. In general the chlorides of aluminium, gold and ferric iron, and the salts of sodium, were found to be most effective. Just what role the electrolytes may serve, whether as an additional stimulant, or in some other, more specific way, is not clear. Hypertonic solutions of "tap-water", or of glucose, produce like results to a lesser degree; but they act apparently by increasing the osmotic contration of the medium, so that their effect is probably not a specific one in the chemical sense. The result produced by any one of these reagents is to augment the intensity of conjugation (increase the proportion of conjugating animals), for a small percentage of individuals may conjugate in a culture to which water alone has been added (in place of salt solution), or sometimes even in the main culture which had been renewed with hay infusion.

In the light of this recent work in which refined methods of experimentation were employed successfully with one race, the question has been asked whether we cannot by such means induce conjugation in any race of Infusoria. As applied to Paramoecium in particular--are there any racial

differences in respect to conjugation, or are the alleged diversities environmental in origin? According to Zweibaum we ought to be able to induce conjugation in any race of Paramoecium caudatum by using those methods which he finds most effective; for he holds that all supposed racial diversities, such as those described by Jennings and Hargitt('10) for size, result from cultural differences. To state his position more clearly, I quote from p.276 of his paper--

> "Dernièrement encore pour les Paramaecies Jennings determine diverses races--injustement à mon avis--et affirme que les conditions de conjugaison diffèrent considérablement selon ces races. L'auteur déplace ainsi le centre de gravité de la question sans ajouter une conception nouvelle et un déterminisme positif dans les considérations de conjugaison chez les Paramaecies".

And again, in summarizing the conditions which he considers sufficient for conjugation in Par.caudatum, he writes(p.292)--

> "Comme conclusion nous pouvons dire que les Paramaecies que Jennings et Hargitt croyent appartenir à des races diverses, se conjugueront toujours, lorsque après avoir passé par un état de disette prolongée de 5-6 semaines, on remplira les autres conditions necessaires--à savoir--la disette comme agent de l'action instantanée, la température de 20-23°C et la composition du milieu (voir 2ème partie).
>
> "Ce qu'on dit être des races diverses de Paramaecium n'est que le résultat de la quantité de la nourriture".

It is apparent from these statements that Zweibaum holds that the conditions for conjugation as worked out for one race of Paramoecium apply to all other races of the same species--at least in P.caudatum;and he strongly in clines toward the view that conjugation is regulated primarily thru environmental conditions. This,at any rate is the view expressed by Enriques (on the basis of Zweibaum's work[1]) for he did not see fit to change his original point of view, believing that the preliminary period of semi-starvation, of 5 to 6 weeks,to which the Paramoecia were subjected was but a step in the experimental procedure,making the organisms more susceptible to the effects of the salts.

The present study was undertaken mainly with this object in view:to ascertain whether the so called "conditions for conjugation" worked out for one race of Paramoecium are effective for other races,and in general,to determine so far as possible the differences in the conditions required to induce conjugation in different races. An attempt was made, also, to find out whether the various races under observation were characterized by different time intervals between conjugation periods,or as we might say,by diverse "sexual periodicities". Jennings ('10) called attention to such apparent periodicities in certain races of P.aurelia. His studies on P.caudatum,while less extensive,indicated that in some races of this species conjugation occurs at intervals of several

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1. See Zweibaum ('12),footnote by Enriques,pp.346-347.

months,or perhaps years. The importance of further work relative to this point was felt more keenly in view of the greater perfection attained in the experimental (or environmental) study of conjugation. Even in other groups of organisms the study of natural periodicities in respect to sexual activity—apart from environmental influences--has not received the attention which it merits.

This work was undertaken at Dr.H.S.Jennings' suggestion,with this end in view. I wish here to express my thanks to Professor Jennings for his kindly advice and criticism,as well as for assistance in the procuring and rearing of material for cultures.

For our purpose then three general questions should be kept in mind. (1) Do there exist differences between various races of Paramoecium with respect to conjugation? (2) If so,what are they? (3) From a comparative study of different races of the same species,and of the different species,what can be said in general regarding hte nature of the conditions for conjugation in the Infusoria?

Any answer to the third question must depend,in part at least,upon the facts brot out in connection with the other two. If constant natural differences in the time interval between successive epidemics of conjugation in different races and species can be demonstrated,it would coincide better with the view that conjugation is primarily a function of some internal factors,altho of course it would not exclude the setting in operation of these factors by environmental conditions. The complete subordination of conjugation to direct environmental conditions might lead us to regard the

process as mainly environmental in its origin, and perhaps directly adaptive in its functional significance.

## MATERIAL AND METHODS.

Twenty-four races of Paramoecium, each derived originally from a distinct individual, were used in the course of this work; eleven representing P. caudatum, and thirteen, P. aurelia. Many other races were isolated, but owing to unfavorable culture conditions died out before reaching the "mature" condition of old cultures.

This dying out of unbalanced cultures is conditioned largely by the racial characteristics of the protozoan isolated, it being ill adapted to the conditions of growth supplied in the laboratory. This is shown by the fact that other races which were reared with difficulty to the condition of stable cultures in the beginning were found to show, some months later, this same inability to develop rapidly when isolated individuals were reared again under rich culture conditions. Deleterious strains of bacteria undoubtedly serve as the immediate cause of the death of a culture in most cases, but the diversities between races of protozoans as regards resistance must be taken into account in order to understand why one succumbs in an environment in which another grows and apparently thrives. If two races distinct in size, one of Par. caudatum, the other of aurelia, are reared together in the same culture dish, hence in the same medium, one may survive, the other die, or both may live together. Like differences undoubtedly exist between races of the same species; for my work

indicates thatsuch races show constant natural differences as regards their resistance to specific reagents and their ability to grow readily in slide cultures as well as in mass cultures.

During the course of development of these cultures account was taken of races under similar growth conditions in order to determine in advance which ones were subject to periodic epidemics of conjugation,and the nature of these rhythmic changes,so as to compare their behavior under usual conditions with that to be obtained later in the experiments. Certain cultures were subjected to daily observation for weeks at a time with this object in view,and record kept of sporadic occurrences,and of conjugation en masse. This revealed what races were subject to frequent epidemics,and in what ones conjugation was rare or lacking. Most of these observations were made daily under a binocular microscope of wide field of vision,upon cultures growing in Stender dishes measuring 5 cm.,6 cm.and 10 cm.in diameter--so that any but rare conjugating pairs could hardly be overlooked. The same method of daily observation was adopted during the course of experiments with small cultures,and since these cultures were comparatively free from sediment it was an easy matter to conduct thoro observations upon them during the few days required for each experiment.

Many of the experiments referred to in this paper were carried out after the procedure recommended by Zweibaum (see p. 3.),using salt solutions,with the object of showing whether each race would not yield conjugants when the conditions which he found most favorable were supplied. For this

purpose sub-cultures were derived from the main cultures by diluting 50 to 100 cc. of the culture containing many Paramoecia with an equal part of distilled water. These were allowed to stand for several weeks as"dormant cultures",with very little nutriment,at the end of which time hay infusion was added. Such cultures to which hay infusion has been added to induce rapid multiplication will be referred to as "renewed cultures". After about the fifth day portions of these cultures were treated with salt solutions in small Stender dishes.

The renewed cultures of P.aurelia often yielded about as many conjugating pairs as the smaller (experimental) cultures derived from them,and treated with salt solutions. It was found further that certain inorganic salts added to the renewed cultures directly,i.e.immediately following the period of dormancy,at the time of renewal, made these organisms (P.aurelia) more susceptible to conjugation after the preliminary period of multiplication of about five days. Small watch-glass cultures derived from these usually yielded conjugants earlier--after the third or fourth day following renewal of the main culture,--but the renewed cultures themselves often yielded just as many pairs after the fifth day, showing that the sudden change brot about thru such "isolation experiments" is not an essential condition for the appearance of conjugation.

In general it may be said that large cultures of Paramoecium,of either species, give fewer conjugating pairs than small cultures of the same race when treated in a similar manner. This condition may perhaps be attributed to the

greater accumulation of organic matter (excretion products) in large cultures than in small, and in part to the more uniform conditions.

The experimental procedure will be described in greater detail in connection with the experiments themselves. The culture medium used thruout during the course of this work on Paramoecium consisted of hay infusion. "Tap-water" was used to maintain the stock cultures, which were contained in battery jars and kept at room temperature. Cultures intended for use in future experiments were diluted with distilled water just before being subjected to their period of dormancy, and at the end of this period were renewed with an equal part of hay infusion in distilled water, changed subsequently every three to four days. So after the multiplication period of five days or longer the organisms to be subjected to the treatment with salts were in essentially a distilled water medium. Spring water from a perpetual spring near the Johns Hopkins University campus was found to be most favorable for maintaining Paramoecium lines on depression-slides (as slide cultures), for the organisms are very sensitive to sudden changes in the character of the medium such as attend this method.

The various strains of distinct origin are designated each by a separate number, usually followed by a small letter. Cultures of the same strain or race derived each from one individual of the original culture (frequently from an exconjugant), are designated by the same number followed by a different letter. Sub-cultures are often distinguished by Roman numerals.

## PARAMOECIUM CAUDATUM

I shall take up first the question of conjugation in races of P.caudatum,because it was in a race of this species that Zweibaum worked out the conditions which he claims to be equally applicable to any race of this species. For if his conclusion is valid it should be possible to repeat his experiments upon various other strains under parallel conditions,and thus show definitely that there are no inherent diversities with respect to conjugation. On the other hand,if two races,which have had the same environmental history,react differently under these same experimental conditions,one giving conjugants,the other not,--or even if they react differently to other conditions which have been shown to favor conjugation in some one race,--then we have evidence for racial diversities.

The following series of experiments with three races of P.caudatum are illustrative of others which were carried out at other times with the same races under less parallel conditions. Experiments with salt solutions found by Zweibaum to be most effective in his work,were conducted upon each strain,on the same days (table I). Cultures 6aII and 5aII had been dormant since Mar.28.1919. A culture 17aII (containing also individuals of a race of P.aurelia,13a) had been dormant since Apr.5. On May 8 the first of these cultures was renewed,and on the following day the other two cultures. Experiments were set on May 13 from samples of each culture,using $NaNO_3$ and $FeCl_3$ solutions;and these gave, in the case of 5aII and 6aII no conjugants,in 17aII,however,

conjugation to the extent of 30-50% (May 14). Conjugants were found in smaller numbers (2-5%) in the renewed culture of 17aII to which no salts had been added, so that the results obtained with this race are clearly comparable to those obtained by Zweibaum in his experiments: the effect of the salts is to augment the intensity of conjugation,

This same race had shown its ability earlier to yield conjugants, when another culture, 17aI, was renewed after about ten days of semi-starvation (conjugation on Feb.26). Similar results were obtained with a third culture of this race, 17aIII, on Apr.10 and 11, when renewed after fifteen days of dormancy. Other cultures of 17aIII (containing also individuals of P.aurelia) likewise gave conjugation (table II).

That the negative results obtained in the above experiments with cultures 5aII and 6aII cannot have been accidental (resulting perhaps from unfavorable culture conditions) seems certain from the fact that similar experiments upon other portionsof these cultures, renewed after longer or shorter periods of dormancy, all proved negative (table I). The further history of these two races may also be referred to in support of this view. Both were isolated from the same abandoned hay culture on Feb.2,1919, and were grown under similar conditions from the start (i.e. as stock cultures). No sign of conjugation was observed in either race during the first six months of their history, altho frequent attempts were made to bring it about by means of isolation experiments, as well as thru the experiments with salts described above. The main cultures were allowed to go dormant from about the middle of July until October 15,1919, on which day

they were renewed. On the twentieth a few conjugating pairs (3-4) were observed in *6a*,and again on the twenty-second. Not one was observed in *5a*, Attempts were made (on Oct.17 and Oct.22) to induce the occurrence of conjugation in large numbers,by subjecting sub-cultures to the action of .00002 N $FeCl_3$ and of .001 N $NaNO_3$ solutions,but the results were negative. It should be remembered,however,that these animals taken from stock cultures had been growing in a medium made up with "tap-water",and so perhaps might not have responded in the same way as animals in a pure aqueous medium when treated with salts. These same stock cultures were then allowed to undergo another long period of dormancy (which may be dated from Nov.1,1919). Two sub-cultures taken from these stock cultures and renewed with spring water and hay infusion on Dec.12,gave in the case of *5a*,one pair of conjugants,in *6a* ,a considerable epidemic of conjugation (1-5%) extending over a period of thirty-three days. Attempts to augment the intensity of conjugation in the latter culture by treating samples with different concentrations of $FeCl_3$ (.00002 N, .00004 N and .00008 N),with $KCl$ (.00025 N and .0005 N) and with $NaNO_3$ (.001 N) were ineffective;that is,the relative number of pairs obtained in these experiments was not greater than in the renewed culture.

A quite different state of affairs was found to ensue during the later history of *17a* . As pointed out above this race proved susceptible to conjugation during its early development. Cultures *17aI,II* and *III* taken at different times from the same stock culture (which was started on

Jan.17,1919 from one individual) yielded conjugants very readily when renewed after two or three weeks of semi-starvation (tables I and II). The general effect of adding salts to the medium was to intensify conjugation in accordance with the results of Enriques and Zweibaum. After four months of cultivation,however,no more conjugants were obtained in the experiments with this race. The culture *17aV*,derived from *17aIII* (which had been continued as the stock culture), when renewed on June 28 after lying dormant for one month, gave no conjugants,and experiments with salt solutions ($FeCl_3$ and $NaNO_3$) such as were found to be effective in earlier experiments with this race,gave negative results. A sub-culture,taken from the main culture *17aIII*,after it had lain dormant for two months,when renewed on Sept.19 gave only one pair of conjugants. The main culture itself (*17aIII*) renewed on Oct.15,after three months of dormancy,gave no conjugants, altho samples were treated,as in the case of *5a* and *6a* (p.14), with solutions of $FeCl_3$ (.00002 N) and $NaNO_3$ (.001 N).

Another set of experiments was conducted with these three races,after more than a year of cultivation,during the month of March,1920. A sub-culture was derived from each of the stock cultures,*17aIII*, *6aI*,and *5aI*, which had been maintained in a dormant state since October,1919,and these were renewed on Mar.18 with distilled water and hay infusion. A good growth of Paramoecia followed. Daily examination of the renewed cultures revealed no conjugants in *17aIII*, a single pair in *6aI* (on Mar.25),and about 1-5% in *5aI* between Mar.22 and 27. The race *17a* is thus shown to be non-susceptible as before,race *6a* much less susceptible than it

had been during the month of December,1919,whereas *5a* conjugated more readily than at any other time during its history. Experiments with salt solutions were found to give essentially negative results. Three experimental cultures of each race were treated respectively with $NaNO_3$ ( .001 N ),$AlCl_3$ ( .00003N) and $FeCl_3$ ( .00002 N ) on Mar.23; and another set was rteated similarly on Mar.26. The treated cultures of *17aIII* gave no conjugants,and those of *6a* and *5a* no more than could be found in the renewed cultures themselves.

Parallel experiments were likewise conducted with other strains of Par.caudatum. In the following series of experiments seven races (other than the ones considered above) were employed (table III). Each had been cultivated under like conditions for one month ,during which time conjugation occurred in culture *2a* on Nov.8 (5%),but not in any of the other cultures. On Nov.14,1919 a dormant culture was started from each of these continuous cultures by diluting 100 cc. of culture with an equal part of distilled water,a few pieces of solid hay being added to each to prevent starvation of the Infusoria. These seven dormant cultures were renewed with hay infusion and distilled water on Mar.3,1920,changed on Mar.6 and Mar.8. On Mar.9 conjugants were found in one of these renewed cultures,*2a*, to the extent of about 5%; on Mar.12 in culture *44a*, 5-10%; and on Mar.17 in culture *57a*, about 5%. The animals in *44a* continued to conjugate in increasing numbers after the twelfth,reaching a maximum of 30—50% on the fifteenth. A similar,but less extensive,epidemic manifested itself in *57a* during a period of many days, attaining a maximum of about 10%.

Experiments with various salt solutions were started on Mar.9, and repeated on Mar.12. In the first set of experiments, in which $FeCl_3$ and $AlCl_3$ were used in the optimal concentrations recommended by Zweibaum, negative results were obtained (Mar.10). In the second attempt, $AlCl_3$ and $NaNO_3$ were used, the first one in two concentrations,—and this time also essentially negative results were obtained, for altho conjugation was already in progress in the culture *44a*, the relative number of conjugants in the experimental cultures was not appreciably altered as a result of the treatment with salts.

If these seven cultures be compared with the continuous cultures of the same races from which they were derived originally, it will be seen that the conditions in one set tend to parallel those in the other; that is, conjugation tends to occur in two distinct cultures of the same race even after they have been subjected to quite diverse conditions. For in the continuous culture *44a* (after the dormant culture had been derived from it on Nov.14) conjugation took the form of an extensive epidemic between Nov.16 and Dec.15; and in the continuous culture *2a* conjugation occurred on Nov.8 (5%), and again, in small numbers, on Jan.11 and 12, 1920. No conjugation was observed to occur in the continuous culture *57a*, but in a sub-culture of this race, which had been set aside on Oct.31 with abundant nutriment, and allowed to undergo evaporation (method of Hance, '17), conjugation occurred to the extent of 10-20% on Nov.7, after about one-fourth of the culture liquid had evaporated. Another observation indicates this race was very nearly as susceptible as *44a* when the conditions were not maintained uniform. Four lines of this race which had been cultivated as isolation cultures on

depression slides from October,1919,until Feb.6,1920,after being merged to form a mass culture gave conjugation on the seventh day (Feb.13).

Further consideration of the development of these races from the time of their isolation is instructive,for in two of them there was observed a periodic recurrence of conjugation under more or less uniform conditions of growth. In one race,38a,started as an exconjugant on Mar.25,1919,it occurred on May 5 (10%), and in the second "generation" (i.e.the exconjugant race 38b,started May 5) on July 14,in smaller numbers (1-5%). No subsequent epidemic of conjugation has been observed in these cultures. The two races 28a and 39a were started on the same day as 38a, each from an exconjugant,and were grown under like conditions;but in neither of these races has conjugation been observed during the first four months when the cultures were regularly examined,nor subsequently when examined only at intervals. Negative results were obtained with race 39a, as with 38b, in the experiments referred to above.

In the other "periodic" race referred to(44a,b,c and d),conjugation has been repeated four times. The original culture 44a was started from an exconjugant on Sept.25,1919, and gave rise to an extensive epidemic on Nov.16,which lasted until Dec.15. This involved practically all individuals in the culture,for as many as 5-10% of the Paramoecia were in union at any one time during most of this period. In three out of four cultures of 44b, started from corresponding exconjugants of 44a on Nov.20,1919, a large epidemic occurred as before,between the dates Jan.30 and Feb.23,1920.

Several new cultures, started on Feb.2 from exconjugants of 44b, were collectively designated as 44c; and in two of these likewise conjugation occurred, making its appearance on Feb.28 in one, on Mar.2 in the other. It made its first appearance in several exconjugant strains of the fourth "generation" (44d, isolated on Feb.28 from a culture of 44c) as early as the twenty-third of March. In the original culture 44a, after the occurrence of conjugation during November and December, no more conjugants were observed until Mar.12, 1920, when they were found to the extent of about 5%. Casual pairing occurred subsequently during a period of several days.

It is important to compare the reactions of these animals of race 44 with those of race 38 during their respective periods of conjugation. In the latter strain a slight disturbance in the culture conditions (addition of hay infusion) caused immediate cessation of the process, whereas in the former it exercised but a temporary and partial influence when several times repeated. We have, as it would appear, two strains in which conjugation tends to manifest itself periodically, in one of which it is largely influenced thru the environment, in the other more nearly independent.

The two races 43a and 57a were derived as exconjugants from the same source as 44a, on the same day (Sept.25, 1920). They were cultivated under parallel conditions and examined regularly during a period of six months, but no conjugants were observed in either of these continuous cultures grown under the same conditions as 44a. During the course of the experiments described above, conjugation occurred in the renewed culture of 57a but not in as great numbers as in 44a;

whereas no conjugation took place in 43a after renewal.

In the remaining race, 8a, used in these experiments, conjugation has never been observed, either in the experiments, or in a contimuous culture maintained under the same uniform conditions as the continuous cultures of the six other strains (listed in table III).

It is apparent from the results of these observations and experiments with several strains of Paramoecium caudatum that no general directions may be formulated by which we can bring about conjugation in a given strain at any one time. When various strains are compared under the same set of conditions they respond differently: in some conjugation occurs, in others it does not. Only one of the strains (17a) used in my experiments may be regarded as being similar to the strain which Zweibaum employed in his studies, and it responded to experimentation only during the first five months of its history. Other strains were found to conjugate readily in cultures which were renewed with hay infusion, after they had undergone a period of dormancy, but the use of salt solutions was not found to cause any appreciable increase in the relative number of conjugants, altho these same solutions had been foud effective in augmenting the intensity of conjugation in 17a.

Others, moreover, persistently failed to give conjugants under uniform and under experimental conditions. Of these, certain ones (39a, 43a) were derived originally from exconjugants, and so might perhaps be regarded as conjugating strains; but the race 8a has never been seen to conjugate--altho it has been under regular observation only during

a period of six months. It is possible that these strains may in time become more susceptible to conjugation. Such a condition is indicated in the two races 5a and 6a, in which, after a period of about eight to ten months of cultivation, conjugation was found to occur in sub-cultures renewed after dormancy. Previous to this time cultures had responded negatively to the same treatment.

Taking the evidence as it stands, we would be forced to conclude that some at least of the strainsstudied here are heritably diverse. Tested by the experimental methods of Zweibaum, which he claims would be effective for any given race of P. caudatum, I have found consistent diversities between races as regards conjugation. My observation upon "periodic" races, moreover, support this same general conclusion. It would be possible to point out other differences between the various races which I have studied—e.g. as regards size, form, division-rate, etc.—but since we are concerned here mainly with differences in respect to conjugation, I shall not attempt any such comparisons. Differences in respect to size and fission-rate have been studied in other races by Jennings and Hargitt ('10), but according to Zweibaum these supposed racial diversities result from differences in the amount of nutriment. Supposing, however, that acquired diversities may arise in different cultures as a result of differences in environment, these same cultures should, in Zweibaum's opinion, yield conjugants after being subjected to the prolonged experimental procedure which he recommends. But this they fail to do in all cases, as the results of my experiments indicate. Cultures which are known to have a common ancestry

(various cultures of the same pedigreed stock, descended from one original individual) do respond similarly in these experiments. But when races of diverse ancestry are compared they almost invariably respond differently. Two races coming from the same habitat (as from the same pond or stream, aquarium or hay culture) may sometimes respond similarly under the same conditions, but as a rule they respond differently. Thus, the two races 38 (a and b) and 39a were from the same general source, a pond near Baltimore, but the first underwent two epidemics of conjugation in the laboratory, during which time no conjugation has been observed in 39a. Similarly the two races 44a and 43a were isolated from the same lot of material collected in a river. In 44a (and its derived cultures) conjugation has occurred in epidemics of long duration, under essentially uniform conditions, whereas in the latter strain (43a) no conjugation has been observed since the beginning, under uniform conditions or under the altered conditions of growth which favored conjugation in 44a (as when cultures are renewed after dormancy).

# SUMMARY OF OBSERVATIONS AND EXPERIMENTS ON PAR.CAUDATUM.

1. There are diversities amongst the various strains of this species as regards their tendency to conjugate under natural and experimental conditions.

2. Some of these diversities in response under a given set of conditions may be qualitative,as illustrated by the different behavior of two conjugating strains when treated with a particular reagent. That is,the conditions for conjugation in all strains are not identical.

3. That the several strains in question are racially diverse is supported by the fact that conjugating strains sometimes maintain their ability to conjugate freely for several "generations",in new cultures started from exconjugants of a previous epidemic.

## PARAMOECIUM AURELIA

My studies upon races of this species open up problems essentially like those presented by caudatum. Some of the cultures were subjected to daily observation during the first weeks of their development. Conjugation was thus shown to be of frequent periodic occurrence in some, rare and apparently sporadic in others,while in some it was found neither during this period of daily observation nor on subsequent examination at intervals of two to five days for several months. At intervals dense watch-glass cultures, derived from the main cultures,were set aside in moist chambers and examined the following day for conjugating pairs. In such cultures the nutriment is quickly used up by the Paramoecia,and the conditions are right for an epidemic of conjugation,provided the organisms were taken from a flourishing culture (method of Maupas,'89). In this manner some of the races which showed no conjugants in the stock cultures were made to give several in these isolation experiments; while others,which showed small numbers in the stock cultures,yielded as many as fifty per cent.in the experiments. As a rule pairing took place earlier,and persisted later, during a series of such experiments than in the main cultures themselves,when the experiments were begun sufficiently in advance of an epidemic of conjugation in the main culture. That is,conjugation could generally be obtained in the dense watch-glass cultures several days before any pairs were seen in the stock culture from which they had been derived, and could be obtained for days after it had ceased in the stock

culture. The effect in such experiments is,then,to augment conjugation in cultures of organisms already in a state of susceptibility. Certain races,however,failed to conjugate during long periods (several months) even when placed under the more favorable conditions mentioned above (isolation experiments). In this connection,also,solutions of various salts were added to some of the watch-glass cultures when isolated. This served to increase somewhat the percentage of the conjugants in certain more susceptible races,but many that did not conjugate still remained.

From the above it will be seen that amongst the various strains of Paramoecium aurelia there appear to be several intergrading conditions as regards conjugation; in some races it occurs spontaneously,in others its occurrence is conditioned (in the laboratory) by certain experimental procedings--alterations in environmental conditions. The question arises whether we cannot by still other ,perhaps more indirect,methods bring about conjugation in all races of this species. So far as the results of my work go,the question may be answered in the affirmative. Of the thirteen races of P.aurelia studied in the beginning,all proved amenable to indirect methods. Six were found to be conjugating races at one time during their existence; that is,they conjugated under more or less uniform conditions of cultivation. Seven were found to be more refractory,yielding only to indirect methods.

The most effective procedure for these particular races was to subject them to a period of semi-starvation lasting from four to eight weeks,then to bring each to astate

of rapid multiplication by adding hay infusion containing certain electrolytes to the culture. The culture medium used most effectively contained $NaNO_3$, final concentration about .001 to .004 N, and a smaller proportion of $Ca(NO_3)_2$, (.00005 to .0002 N). The solution used in later experiments contained $NaNO_3$ alone--and this gave about as good results. It was thot that the calcium used in these preliminary experiments would offset the toxic effect of sodium, and so lead to better growth in the culture, but it was found that the sodium salt when used in low concentrations (.001 to .003 N) was relatively non-toxic to most races of P. aurelia. As will be shown later, the effect of sodium salts upon Paramoecium is to stimulate growth and thereby accelerate the fission-rate.

The period of semi-starvation to which it was found necessary to subject each culture differed considerably, depending upon the race employed. Some few of these (14a, table V, and 16a) proved to be very refractory, for they failed to give conjugants except after fasting for eight to nine weeks. Others, on the other hand (e.g. 13a, table IV, and 25a), conjugated after shorter periods of dormancy---about three to four weeks. The duration of this period serves perhaps as the best criterion for judging of the relative susceptibility of various races toward conjugation, for experiments with salt solutions generally give negative results unless the organisms have become susceptible to conjugation previously thru dormancy.

The effects produced by using salt solutions directly in experiments designed to induce conjugation (method of Zweibaum) are rather variable and unpredictable. As a rule,

the salt solutions increase the percentage of conjugating pairs, but I suspect that their action is largely osmotic rather than chemical. When added to the main cultures when renewed, however, the effect of sodium salt is more marked,-- altho even by this method a relatively small number of conjugants is obtained. As examples of this effect may be cited experiments with race 13a, table IV; 14a, table V; 11i and 11j, table VI; and 3d, table VII. The reactions of these various races, however, may be contrasted with that of race 1b, table VII, in which conjugation is not favored by the presence of sodium nitrate, or if so, only by a lower concentration than that which was found most effective in the other races.

In order to bring out more clearly some of the racial differences between the various strains of P. aurelia, the following observation are recorded. They indicate, also, something regarding the nature of the "periodicities" in conjugation so characteristic of many strains of this species.

Par. aurelia, 1 (b and c). This race was descended from a single individual isolated Jan. 30, 1919, from an old laboratory hay culture. The original culture, 1b, developed very rapidly under laboratory conditions, the animals proving to be quite hardy at all times during their history.

A watch-glass culture containing numerous individuals was set aside on Mar. 17, and on the following day showed conjugation involving about 10% of all. Observations failed to reveal the occurrence of the process in the stock culture previous to the nineteenth, when one or two pairs were noted. It was recorded at intervals during March and April,

but on each occasion in very small numbers (involving usually less than one per cent.). During a period of thirty-two days (Mar.17 until Apr.18) very frequent recourse was had to isolation experiments, in watch-glass cultures, in order to determine whether the organisms continued susceptible. Every one of these experiments yielded conjugants, in numbers ranging from about 5% to as high as 50 or 60% in some instances. Thruout most of this period no conjugation was noted in the stock culture, either on the day preceding each isolation experiment, or on the same day. It frequently happened that in a dense culture set aside in a watch-glass conjugation set in almost immediately, so that at the end of one-half hour 10% of the Paramoecia would be found in union, altho no pairs had been seen in the beginning. Other races responded similarly in these experiments, but not so quickly, nor in such large numbers.

The strain 1b was subjected to experimentation on Sept.30,1919, when renewed cultures were started in small Stender dishes with material taken from the stock culture. This had been allowed to stand for over three months without renewal, altho considerable hay was added to it previously. The animals were found to be in a very healthy state, showing no effects of starvation, altho they were not dividing. Practically no conjugants had been observed in the culture since May 1919, when casually examined. On the second day after renewal conjugation was obtained (on Oct.2) in the culture 1b-A to which no electrolytes had been added. In the other cultures, containing $NaNO_3$, pairing took place in smaller numbers (table VII).

As was pointed out above (p.27) the reaction of these organisms to the presence of NaNO3 in the renewed culture is in marked contrast with that of the other races to be described later, for which a low concentration of this salt acts as a stimulus to conjugation. The animals of culture 1b (and of 1c) always conjugated most readily in an aqueous medium, whenever they conjugated at all. In the stock cultures the Paramoecia conjugated little or not at all, but they remained susceptible during long periods of time, yielding conjugants readily in watch-glass cultures isolated from the main cultures.

Par. aurelia, 3a. The culture was started Jan.23, 1919, from an individual isolated from the same abandoned hay culture as 1b; and so it is not known with certainty whether it represents a race distinct in descent from 1b, altho its characteristics are sufficiently distinct to render this probable. Conjugation was observed in this culture while still small on Feb.13, in about 20-30% of all, and again on Feb.15 and 17 in smaller numbers (3-5%). Conjugants were again seen in this culture on March 12, about one per cent., and later between March 17 and 22, in no case in more than casual numbers. Isolation experiments continued to yield them, however, often as many as five per cent., from Mar.15 until Apr.18, when the experiments were discontinued.

Culture 3b was derived from an exconjugant of 3a isolated on Feb.13. One or two pairs of conjugants were seen in the culture on Apr.7, after long search, while isolation experiments yielded them in small numbers (1% or less) between Mar.19 and Apr.10. Except for the casual pairs noted

above (Apr.7) the stock culture showed none from its beginning until the time when regular observations were discontinued.

Cultures *3c*, *3d* and *3e* were started on March 20 from three exconjugants of *3b*, and represent thus the third "sexual generation" of the race. Each was cultivated under uniform conditions and observed regularly. Conjugation occurred to the extent of 40% in a watch-glass culture derived from *3d* on April 16,but was not discovered in the main culture during the entire period of daily observation (from Mar.20 to Apr.20), nor on subsequent examination at longer intervals. Isolation experiments set before and after the sixteenth (on Apr.15,17 and 18) gave no conjugants. The two related cultures, *3c* and *3e*, failed to show any conjugation, even in the isolation experiments,which were continued until April 20.

Comparing this race,number *3*, with race *1*, it will be seen that in both conjugation occurred naturally under the more or less uniform conditions afforded in large stock cultures. In culture *3a* there is at first apparently a periodicity marked by "epidemics" in the occurrence of the process at intervals of about four weeks. In culture *3b*, started from an exconjugant,the first epidemic appeared after about five weeks; and in *3d*, similarly related to *3b*, after about four weeks. Conjugation in race *1b*, while not occurring at periodic intervals in the stock culture,was readily evoked in isolation experiments during the entire period when such experiments were regularly conducted (Mar.18 to Apr.18). The same irregularity tends to manifest itself in the culture *3a* after the onset of the second period of conjugation (Mar.12).

This irregularity in the occurrence of conjugation during the later stages of growth in a culture in which it has already taken place, in the form of a preceding "epidemic", makes it impossible to measure accurately the interval between conjugation periods except with cultures started from exconjugants, and in which conjugation has not previously occurred. Even the first epidemic of conjugation in a newly developed strain may show two separate phases (see observations on races 11c and 11f, p.33) suggesting that diverse lines, showing acquired differences in susceptibility as regards conjugation may have arisen amongst the offspring of one individual. Later irregularities in conjugation--after a previous epidemic in the same culture--may perhaps be accounted for in part, also, as due to hereditary differences established thru conjugation, among the exconjugants of the first epidemic. Thus diverse strains, with different degrees of susceptibility, each having perhaps a somewhat different periodicity, might be perpetuated in the same culture--coming originally from the same race. If this be true a new clone derived from a single exconjugant, would presumably give conjugants in a more sharply defined epidemic than a culture containing the offspring of several exconjugants, altho originally from the same strain.

Some such interpretation as this may account for the irregular occurrence of the process in the later history of the mass culture 3a, for a study of the later generations (3b and 3d) shows that conjugation in these, like the first epidemic in 3a, is sharply defined. Altho no sufficient evidence is here afforded that hereditary

differences exist between the corresponding cultures of the same generation of exconjugants (3c, 3d and 3e) in respect to conjugation, some observations made upon exconjugant lines of the race 44 (P. caudatum) indicate that such heritable diversities do arise in this manner (p.50).

Par. aurelia, 11a, was obtained as a mass culture on Feb.15,1919, from Professor Jennings, by whom the race had been reared from one individual isolated Jan.18,1919. Conjugation had already been observed in this culture on Feb.5, and again on Feb.15, involving at each epidemic a fair percentage of individuals. On March 5 and 8 pairing was observed in about 10% of all; and from Mar.10 to 12 its occurrence was noted as casual. Conjugants were seen again, in smaller numbers (about 3%), on April 4, altho casual pairs were found as early as Mar.29, and later, on Apr.9. The process, it will be seen, is becoming more irregular in occurrence as time progresses, the separate epidemics becoming much less distinct and the percentage obtained at any one time falling off. This is evidenced, also, by the fact that, altho conjugants were not discovered in the main culture between Mar.12 and 29, yet isolation experiments continued to yield them in considerable numbers (5-30%) between these dates. Three, and possibly four, successive epidemics are thus indicated for race 11a. Results of later observations (in races 11c and 11f, p.33) showed that the occurrence of two sharp epidemics close together in point of time, such as those of Feb.5 and Feb.15, or Mar.5 and Mar.8, may very properly be regarded as one, the first onset being interrupted by an abrupt change in nutritive conditions (the addition of hay infusion to the culture, etc.).

Cultures 11b, 11c, 11d and 11f. These were derived from corresponding exconjugants of 11a isolated on March 20. A well marked epidemic of conjugation, involving about three fourths of the culture, occurred in 11c on Apr.4. The process was repeated on Apr.14 (about 5%), and continued until Apr.18 (casual). In culture 11f conjugation appeared first on Apr.15 (casual), and on the day following, to the extent of about 60%. On Apr.17 about 10% were in union. No conjugants were observed in 11b and 11d, examined daily until Apr.18, and casually therafter.

Exconjugant strains of 11c were started from ten pairs isolated Apr.4; and of 11f, from six pairs, on Apr.17. The twenty exconjugant cultures of 11c, designated as races 11g (1 to 20), were grown under very uniform conditions, in hay infusion made up with distilled water, and renewed each week. Under the favorable conditions thus imposed no conjugation was observed in any of the cultures for over two months. Similar negative results were obtained with the twelve strains derived from 11f exconjugants, designated as 11h (1 to 12), subjected to daily observation for six weeks and examined casually afterward.

On the sixth of July an individual of 11g (from a culture which had lain dormant for about a month) was isolated in a watch-glass, in hay infusion containing .003 N $NaNO_3$. On July 10 conjugation occurred amongst the numerous progeny to the extent of 15%. From the exconjugants two daughter strains were started (11i and 11j). No conjugation was noted in these cultures prior to July 24, when they were left unobserved and unaltered.

In each of these conjugation was again obtained in small numbers during September by renewing the dormant cultures with hay infusion containing NaN03 (table VI). No conjugation occurred,however, in those cultures which were renewed with hay infusion alone.

The study of 11a and its descendants reveals a condition which is prevalent in various other races of this species (e.g. 3a and 23a),as well as in caudatum. Conjugation was found to occur very readily in cultures of the race during the first few months of its history,usually under fairly uniform conditions; whereas later the process could be evoked only after renewing cultures which had undergone dormancy. The natural periodicity which characterized the early stages became suppressed,and the relative number of conjugants obtained likewise fell off considerably as time progressed. The effect is probably not due to unfavorable culture conditions long maintained,altho this may play some part,nor to close inbreeding; but is probably connected in some way with the adjustment of the organisms to a state of continuous,rapid multiplication. This point will be treated more at length in the discussion (p.43).

The eight races designated 13a, 14a, 15a, 16a and 23a, 24a, 25a, and 26a were derived on Feb.28,1919 from the same source--an abondoned hay culture containing various infusoria. Each was descended from a single exconjugant,the original individual of 13a having conjugated with 23a, 14a with 24a, etc. It is important to compare these races from the start,since all had been subjected to the same environmental influences previously. The cultures were

examined frequently for eight weeks, while numerous tests for conjugation were made with dense watchglass cultures isolated from the main jars. All the stock cultures developed rapidly, except 24a and 26a, in which growth was more tardy, owing in part to overnutrition, to some extent possibly to racial weaknesses. Conjugation occurred in two cultures, 23a and 25a, during the period of continuous cultivation, but not in the six others.

In 23a several pairs were observed as early as Mar. 11, eleven days after the previous union; and again on Mar. 17. They were found in larger numbers (5-15%) from Mar. 18 to 22, but only casually thereafter until Mar. 31, when conjugation practically ceased in the continuous culture. Isolation experiments gave as high as 40% on Mar. 15, and subsequently until April, in diminishing numbers (Mar. 18, 40%, Mar, 20, 30%, Mar. 26, 5%, etc.).

The epidemic in 25a was less intense, involving at any one time a much smaller percentage of organisms, and lasting but a short time (Mar. 18-25). Isolation experiments yielded conjugants during a somewhat longer period, but in scarcely any greater numbers.

The sequence of events in these two cultures is seen to be very similar to that which obtains in such races as 3a and b, 11a and its derivatives. That is, there is a period of vegetative growth lasting from two to three weeks, after which conjugation ensues. The duration of this period of conjugation depends in large measure upon the susceptibility of the races in question, beginning earlier and lasting longer in the more susceptible ones--except in some few

instances where, in small cultures, the process is consummated in one or two days (e.g. 11f).

In order to determine whether these races (23a and 25a) which had conjugated under uniform conditions had really done so as a result of their greater natural susceptibility, and not, as might be supposed, because of more favorable environmental conditions, new strains were started from isolated exconjugants of each. In this way the initial medium was greatly altered, new strains of bacteria coming in, with the elimination perhaps of many strains of bacteria present in the old cultures. Of the two strains derived from exconjugants of 23a which were reared successfully, conjugation occurred in each, approximately three weeks later. In one of these, 23f, isolated Mar.21, conjugants were observed on Apr.14 (5-10%), Apr.15 and 16 (casual). They appeared also in an isolation experiment on Apr.17. In the other culture, 231, isolated Mar.21, conjugation was only casual, one to three pairs being seen on Apr.12 in a mixed culture of two races, P.caudatum 17a and P.aurelia 231 (see table II). Two cultures of 25a exconjugants (25e and 25h) started on the same day as those from 23a, and similarly treated, showed no conjugants, even in isolation experiments. The relative susceptibility of these two races, of the second generation, to each other is thus shown to be the same as for the two parental strains. That is, the race 23 seems more susceptible than 25 in each case, --altho conjugation failed to occur in the second generation of 25, and was less intense during the second epidemic (second generation) of 23 than during the first.

In the six remaining stock cultures (of races 13a, 14a, etc.) no conjugation was found to occur during the first five months while continuous cultivation was resorted to,altho it was obtained by indirect (experimental) methods. Those experiments in which salt solutions were used in the way recommended by Zweibaum gave less striking results in this species than in the one caudatum race,17a, in which they were effective. Compare,for example,the results of such experiments with race 13a (P.aurelia) with those in race 17a (P.caudatum) when both strains were growing in the same culture (table I). As was pointed out on p.25 ,cultures of nearlyall races,when renewed with hay infusion containing $NaNO3$ (.001-.004 N) may give conjugants when none can be found in controls--renewed with an aqueous medium.

The duration of dormancy preceding the growth period is perhaps the most potent influence favoring conjugation,for it was only by renewing cultures which had long been dormant that conjugation could be elicited at all in such races as 14a (table V),and 16a. In these two strains a preliminary period of dormancy of 7 to 8 weeks was required before conjugation could be obtained in the cultures renewed with a saline medium. With other races,e.g. 24a and 25a, only 3 to 4 weeks of dormancy was required before renewal in the same way. One of these (25a),it will be noted,had been found earlier to conjugate under uniform conditions (in the stock culture),altho it had later lost this tendency. It was shown afterward that three months of dormancy was sufficient for conjugation in any of the races of aurelia when renewed with an aqueous medium (tap water),for when the stock cultures

were renewed in October,1919,after this period of dormancy, a few conjugants were seen in all of those cultures in which none had been discovered previously,as well as those in which they had been found before.

## SUMMARY OF OBSERVATIONS AND EXPERIMENTS ON PAR.AURELIA

1. In this species,as in the preceding,there are evidences of diversities in respect to conjugation. Some strains show a marked tendency to conjugate repeatedly,at regular intervals,while others,under the same uniform conditions,do not conjugate during long periods of time. Diversities in response to the same set of experimental conditions are sometimes indicated--as when one race shows a greater tendendy to conjugate in a particular saline medium than in an aqueous medium, another race so treated showing a lesser tendency.

2. These diversities probably have a racial basis,for if two cultures of the same strain,each having had a similar history,be subjected to the same treatment they show about an equal tendency to conjugate. Furthermore,when two different strains are compared they are found to preserve about the same relative degree of susceptibility,under the given set of conditions,as they showed in the beginning.

## GENERAL SUMMARY AND DISCUSSION.

The following conclusions regarding the causes and periodicity of conjugation may be drawn from the observations and experiments above set forth.

1. Various strains of the same species display different degrees of susceptibility,or readiness,to conjugate when taken from a similar environment and grown under like conditions.

2. They may likewise show diverse reactions as regards conjugation when subjected to the same experimental conditions,--as when treated with a certain salt solution.

3. A high degree of susceptibility is indicated by a tendency for conjugation to appear in successive "epidemics" within a given strain,and by the relative number of individuals involved.

4. The observed time interval between conjugation periods in Paramoecium caudatum is from four to twelve weeks. Conjugation was recurrent in culture 38a after six weeks,in 38b after ten weeks. In culture 44a it occurred after seven to eight weeks,and again after twelve weeks,but in the smaller cultures derived from it (44b, c and d) at intervals of about four to eight weeks.

5. The time interval in Paramoecium aurelia is from two to six weeks (see p.30,and p.32).

6. There exist strains which do not conjugate during long periods of time,and in which,were the conditions maintained uniform and favorable for growth,the process might probably be postponed indefinitely.

7. Many of these races which do not conjugate can be rendered susceptible to conjugation by subjecting them to a prolonged period of reduced nutrition,thus inhibiting multiplication. Such "dormant" cultures,of any race,when renewed by supplying abundant nutriment,may yield conjugants more readily (if conjugation occurs at all) than control cultures of the same strain which have been kept under conditions of constant growth,

8. The addition of certain salt solutions to samples taken from these renewed cultures after a few days of growth may give an increase in the relative number of conjugants in certain races,without affecting appreciably the proportion of conjugants in other races of the same species (as worked out especially for Par.caudatum.).

9. A trace of sodium nitrate,added to the renewed cultures at the time of their renewal,serves to augment conjugation in many races of Par.aurelia. Diverse races appear to react differently toward equal concentrations of the salt as used in this way,for race 1b, and its derivatives,underwent conjugation more readily in aqueous medium than in the solutions of the salt in concentrations found most effective for the other races of this species. This same race multiplied more steadily and maintained its numbers longer in aqueous cultures than other races of this species.

10. There is a progressive tendency,probably in all races, for conjugation to become suppressed after prolonged cultivation under laboratory conditions. Evidence for this has already been presented in the body of this paper--

in Par.caudatum,race 17a on p.14,race 38, p.18; and in Par.aurelia,race 3, p.29,race 11,p.34, races 23 and 25, p.36. Races which,early in their history,showed a tendency to conjugate at frequent intervals,and in large numbers at each epidemic,retain their susceptibility longer than those races which at first conjugated only at longer intervals and in small numbers. The susceptibility of such cultures,in which conjugation is in abeyance,can often be partially restored by subjecting them to a long period of dormancy,as described under point 7. Thus,a culture of 23i, renewed on Jan.10,1920, after a five month period of dormancy,gave conjugants on Jan.16. From an exconjugant a new culture,23k, was started, and in this conjugation was repeated under essentially uniform conditions. (In several new exconjugant lines,231, (1) to (6),however,conjugation did not occur.) This shows that the very long dormant period to which the organisms of this race were exposed had restored their power to conjugate periodically,as in the original culture,23a, of this race (see p.35).

It was thot, at first, that this tendency for conjugation to become suppressed might result from long continued growth under laboratory conditions,the organisms becoming weakened,as it were,by the rich artificial medium of hay infusion. We should expect,on this assumption,to find that races which do not conjugate are ill adapted to these conditions in the laboratory. But this is the reverse of what usually obtains. In general,those races which conjugate but rarely,or not at all,under laboratory conditions prove to be more amenable to steady cultivation than conjugating strains; altho the latter,especially during their early

history, may multiply rapidly when the conditions are favorable for growth.

Observations upon other species, and other genera, likewise indicate that the absence of conjugation is associated rather with a more complete adaptation of the organisms to their surroundings. Eight strains of an unidentified Paramoecium [2] (apparently the Par. trichium of Stokes, '88) which were studied for six weeks, seemed to be very poorly adapted to active growth in the laboratory, and were finally abandoned,--yet in six of these eight strains conjugation was extensive. On the other hand, one race of Oxytricha which I have had in the laboratory for over a year, has never been seen to conjugate, altho it was subjected at times to the same experimental procedure (with the exception of adding salts to the renewed cultures directly) as my races of Paramoecium. But this race—as well as two others of Oxytricha, which did not conjugate during two months observation—were found to thrive as well in the laboratory as any other Infusoria which I have cultivated. Similarly, in twelve races

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2. Three other races from another source, resembling closely the eight here described, were found to develop a green coloration after about one month in the laboratory, thus proving their identity with Par. bursaria. Accompanying the increase in the number of algal cells in their bodies, the Paramoecia became larger and much more truncated anteriorly.

of Colpidium colpoda,studied under experimental as well as natural conditions,I have never observed a single conjugating pair,and these cultures grew much better than any of the Paramoecium cultures which I have handled.

The view which I have been led to adopt regarding the final disappearance of conjugation in previously conjugating stocks,is that it is a phenomenon accompanying some particular type of progressive adaptation of the organisms to their environment. Just what the nature of this adjustment may be is less clear,but indications are that it is in some way bound up with fission-rate.

A comparative study of division rate as such,in the various races of Paramoecium here treated,shows that there is no simple correlation between high or low division rate and a strong tendency to conjugate. Amongst those races having a high rate of fission and resembling each other closely in size and appearance,there are those which conjugate repeatedly and those which do not; and the same may be said for the slowly dividing strains.

But it seems probable,in the light of our experimental knowledge,that there is an indirect connection between conjugation and fission-rate. Whenever conjugation occurs in a culture en masse,it usually does so after a period of accelerated division. This relation has been emphasized by several investigators working with many diverse genera of Infusoria,both free living and parasitic. In fact Maupas regarded it as one of the primary conditions preceding a conjugation epidemic in the many species which he studied, and applied it practically in his work.

In further support of this view--that conjugation is conditioned by a preliminary period of increased division--we may recall the effects produced by adding salts to the cultures of Par.aurelia when renewed. In a medium of sodium nitrate (of .001 to .005M concentration) conjugation is almost invariably augmented in this species (Cf.,however,race 1b to the contrary,which conjugated as well in an aqueous medium). Oftentimes conjugation can be elicited in such "salt cultures" when none occurs in the controls renewed with an aqueous medium. It may be shown very easily by means of slide cultures,changed daily,that $NaNO_3$ in such concentrations acts as a stimulant to growth. The division rate in these treated lines may be maintained at a considerably higher level than that of the controls for at least two weeks (table VIII). Some races apparently do not tolerate the continuous action of the salt and die after two or three days of accelerated growth,--and the death rate is greater in any race so treated than in the controls. These observations apply to races of both species of Paramoecium,but only occasionally is a race of caudatum found which will tolerate the action of the salt as used in these experiments.

J.Spek ('19) has conducted similar experiments on Par.caudatum,using various salts in the medium,and finds that many of these--in particular those of lithium and sodium--tend to accelerate fission-rate.

My experiments upon Paramoecium using $MgSO_4$ in the culture medium indicate that this salt has a depressing effect upon growth. Most races become readily adapted to a .01M concentration,and may continue to live for months in

such cultures to which hay has been added. The division rate is not appreciably diminished by this salt, altho extensive experiments may yield more definite results than I have thus far attained. In the few conjugation experiments in which $MgSO_4$ was used (P.aurelia) the results were negative, except when the solution was used indirectly to increase the osmotic concentration of the medium in a derived culture. These cultures, when renewed with such a medium, continued to live, but failed to grow with sufficient rapidity to develop the initial impulse to conjugate (ex hypothese).

Considering now the second condition, or antecedent, to conjugation, namely that of subjecting the cultures to a period of dormancy,--we find few facts to guide us in interpreting the results. I have been inclined to view this phase in the life-history as a period characterizing the major portion of an infusorian's existence in nature. The stages of rapid multiplication such as we find in fermenting hay cultures in the laboratory, are of rather exceptional occurrence in ponds and streams. They represent periods calling for rapid readjustment to new and ever changing situations. At such unusual periods the division rate is probably at its height, and it is conceivable that a regulation in division may supervene in course of time if the new conditions are long maintained. It is a matter of frequent comment by investigators that conjugation occurs most readily in cultures which have but recently been brot into the labotatory and subjected to conditions which favor rapid multiplication. Secondary conditions, such as a sudden alteration in the amount of nutriment, or treatment with salts, may enhance the

possibilities of obtaining conjugation en masse. But the more effective condition for most races is that they shall have multiplied extensively, after a previous period of dormancy. Only occasionally do conditions in nature approximate those in a fermenting hay culture, as when a small pool, containing decaying vegetation, dries up. So that the majority of animals brot into the laboratory from ponds and streams would be in essentially the same resting state as those in a dormant culture in the laboratory.

If any regulation in division rate does occur in organisms subjected to conditions favoring rapid growth, one ought to be able to test it experimentally, using two cultures of the same strain, one of which had lain dormant for several months, the other having undergone continuous growth. Such a regulation has been demonstrated by Jollos ('13) in Paramoecia when subjected to a higher temperature. Lines which had been growing at 19°, after being transferred to 31° divided at a rate of 9 per 48 hr. period; after six months at this temperature, however, at the rate of 7. The preliminary experiments which I have conducted with a small number of lines do indicate that dormant animals are capable of dividing faster at first than those taken from a continuous culture,--and this is in agreement with general observations upon mass cultures when renewed. But more extensive experiments are needed before this relation can be said to be established.

Further support for the view that conjugation is initiated by a period of unregulated division is obtained from the records of fission-rate in strains which conjugate at regular intervals. As pointed out by Hertwig ('89),and fully demonstrated by Jennings ('13),the most apparent immediate effect produced by conjugation is a decrease in fission-rate. This lowering tends to disappear completely in about two months (P.caudatum). My observations on conjugating strains indicate a similar condition which may be repeated after each recurrence of the process; and this has led me to think that the periodicity in conjugation may in some way be controlled by this tendency for fission-rate to increase gradually to a high point. This is the essential idea involved in Hertwig's explanation of conjugation (1889) as a process following a period of unregulated division,altho he recognized also an alternative process of nuclear reorganization ("parthenogenisis") in Par.aurelia. He says (p.226 and ff.)--

> "Für das,was ich beweisen möchte,genügen die Resultate in ihrer jetzigen aphoristischen Form; sie zeigen,dass fortgesetzte Theilungen im Infusor eine Neigung zur Conjugation veranlassen,dass aber ein künstlicher Verhinderung der Vereinigung die Theilungen in lebhafter Weise fortgesetzt werden, bis ein Moment eintritt,in welchem ohne Conjugation ein Ersatz des Hauptkerns durch Abkömmlinge des Nebenkerns eintritt. ------------------------------
>
> "Wer die Conjugation der Infusorien erklären will,muss dem Gesagten zufolge mit 2 Erscheinungen rechnen: 1.fortgesetzte Theilungen ohne Conjugation

> "führen zum Untergang; 2.trotzdem besitzt das Infusor zur Zeit der Conjugation unverminderte oder sogar erhöhte Theilfähigkeit. Wenn wir Bütschli's Theorie zu Grunde legen,stehen beide Sätze miteinander im Widerspruche; sie sind aber sofort in Einklang zu bringen,wenn wir annehmen,dass zur Zeit,wo die Conjugation eintritt,nicht eine herabgesetzte,sondern eine übermässig erhöhte Lebensenergie besteht. Dann hat die Conjugation nicht den Zweck,die Lebensenergie noch weiter zu steigern,sondern die gesteigerte Lebensthätigkeit so zu regulieren,dass sie nicht zur Zerstörung des Organismus führt; sie heilt nicht die durch physiologische Usur entstandenen Defecte, sondern verhindert,dass derartige Defecte durch Uebermass der Function entstehen".

His later observations ('04) on Dileptus are likewise in accord with this earlier expressed view.

The following observations,which were made at various times during the course of my work,have a bearing upon this point. In the race 44a, of Par.caudatum,started Sept.23, 1919,from an exconjugant,the fission-rate was found to attain to a maximum during the period from Nov.15 to Nov.28 (8th. and 9th.weeks) in four lines started in October. And it was at this time,also,that conjugation was extensive in the stock culture of 44a, commencing on Nov.16 and lasting until Dec.15 (8th.to 11th.weeks). From exconjugants of this culture sixteen new lines were started--44b (1) to (8), two lines from each. Both lines of 44b (5) died after six weeks,but from the remaining strains,four mass cultures were derived: 44b(2)

and 44b (3) on Jan.9, 44b (7) and (8) on Jan.21,1920. Conjugation occurred extensively in three of these mass cultures, during approximately the same period of time in each,beginning on Jan.30 in 44b (7) and lasting until Feb.27. Just preceding conjugation,during the tenth week,the fission-rate in the slide cultures reached the highest point which it had attained since the second week following the previous conjugation,as shown by the average number of divisions weekly for all the lines. (This first maximum may possibly represent a compensatory tendency after the drop immediately following conjugation,for the average rate for the first two weeks is less than that for the second two weeks.) New strains were again started from exconjugants of the culture 44b (2) on Feb.2,1920,and were designated 44c (1) to (12), From the six which survived,mass cultures were started on Feb.16, and in two of these conjugation set in at the end of the fourth week. The fission-rate for these two strains attained its maximum during the fifth week. A similar set of experiments was conducted using forty exconjugant strains derived from one of the above cultures,and designated 44d (1) to (40). Twenty mass cultures were started,and in six of these conjugation occurred,setting in during the fourth week in each one. The fission-rate(in four lines of each strain) attained its highest point at about the third week. It is significant in this connection to note that in almost every instance conjugation occurred in rapidly dividing strains. Slow strains, altho derived originally from exconjugants of the same race, do not conjugate,even after a period of three months has elapsed.

These experiments bring out another point which has already been touched upon (p.32). There appear to be racial diversities amongst the exconjugant strains, originally from the same race. When a particular strain shows a tendency to undergo depression, all of its four lines may be affected, and frequently all die at about the same time (usually at the end of four to six weeks),--whereas in the four lines of some other strain no depression may occur at this time.

My experiments with Par.aurelia likewise support the view set forth above: that conjugation occurs at about the time when division is at its maximum, or shortly afterward. Thus, in the strains of 23k (see p.41) the fission-rate was highest during the second week, and conjugation occurred (in one culture) during the third week.

All this lends support to the hypothesis that it is the unusual conditions of rapid growth which favor conjugation. Circumstances which favor rapid growth may likewise favor conjugation. Abundant nutriment, and the presence of reagents which stimulate growth without harming the organisms, act in this way; but for these conditions to operate most effectively the organisms themselves must have come from freely conjugating strains, rendered potentially more capable of rapid division thru dormancy.

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## TABLES OF EXPERIMENTS.

Following is a tabulation of results obtained in experiments designed to induce conjugation in the various races of Paramoecium (aurelia and caudatum). The tables indicate how long each culture was subjected to dormancy, when renewed, and the nature of the medium. Distilled water (and hay infusion) was used except as otherwise indicated. The concentrations of "salt cultures" are expressed as final concentrations for the salts in question.

* The "Na+Ca solution" used in many experiments was composed of 100 parts N sol. of $NaNO_3$ + 5 parts N sol. of $Ca(NO_3)_2$ + 2 parts N sol. of $NaHCO_3$; the dilutions given being expressed in terms of the concentration of the $NaNO_3$.

Conjugation is expressed, when absent as "none"; when present in terms of the percentage involved (approximate). "Casual" conjugation is about one percent., or less, or an indefinite small number.

Unless otherwise stated, the experiments with salts (and other reagents) given in the last column, were conducted—using one part of culture containing numerous Paramoecia and three parts of the reagent.

Table I.

| dormant culture | date renewed; cult.medium | conjug.in renewed culture | sub-cultures 24 hrs.after treatment:conjugation |
|---|---|---|---|
| 5aII<br>Mar.28, 1919. | A.Apr.11 (aqueous) | none | Apr.18,untreated :none |
| | B.Apr.23 (aqueous) | none | Apr.28,untreated : " |
| | | | May 3,+$NaNO_3$(.001N) : " |
| | | | " 3,+$FeCl_3$(.00002N) : " |
| | C.May 9 (aqueous) | none | " 14,+$NaNO_3$(.001N) : " |
| | | | " 14,+$FeCl_3$(.00002N) : " |
| | D.May 28 (aqueous) | none | " 26,+$NaNO_3$(.001N) : " |
| | | | " 28, " " : " |
| | | | " 26,+$FeCl_3$(.00002N) : " |
| | | | " 28, " " : " |
| 6aII<br>Mar.28, 1919. | A.Apr.11 (aqueous) | none | Apr.14,untreated : " |
| | | | " 14, " : " |
| | B.Apr.23 (aqueous) | none | " 28, " : " |
| | | | May 3,+$NaNO_3$(.001N) : " |
| | | | " 3,+$FeCl_3$(.00002N) : " |
| | C.May 8 (aqueous) | none | " 14,untreated : " |
| | | | " 14,+$NaNO_3$(.001N) : " |
| | | | " 17, " " : " |
| | | | " 19, " " : " |
| | | | " 14,+$FeCl_3$(.00002N) : " |
| | | | " 17, " " : " |
| | | | " 19, " " : " |
| | D.May 23 (aqueous) | none | " 26,+$NaNO_3$(.001N) : " |
| | | | " 28, " " : " |
| | | | " 26,+$FeCl_3$(.00002N) : " |
| | | | " 28, " " : " |
| | E1.June 6 "Na+Ca sol." (.01N) | none | June14,3cc.culture+ 1cc.$FeCl_3$(.0002N) " |
| | | | " 14,6cc.culture+ 1cc.$FeCl_3$(.0002N) " |
| | | | " 14,+Na+Ca(.01N) : " |
| | | | " 14, " (.02N) : " |
| | | | " 18, " (.01N) : " |
| | | | " 18,10cc.culture+ 1cc.$FeCl_3$(.0002N) " |
| | E2.June 6 (aqueous) | none | June14,+"tap-water" : " |
| | | | " 14,+$NaNO_3$(.001N) : " |
| | | | " 14,+$FeCl_3$(.00002N) : " |
| P.caud. 17aII + P.aurel. 13a<br>Apr.5, 1919 (mixed) | A.Apr.11 (aqueous) | Apm.12:one pair of caudatum. | Apr.14,untreated : " |
| | B.Apr.23 (aqueous) | none | Apr.25,untreated : " |
| | C.May 9 (aqueous) | P.Caudatum:<br>May 14:2-5%<br>P.aurelia:<br>May 13,casual<br>" 14, "<br>" 16,10-20%<br>" 17, 5-10% | May 14,+$NaNO_3$(.001N):--<br>P.caudatum :20-30%<br>P.aurelia : 5%<br>May 14,+$FeCl_3$(.00002N)-<br>P.caudatum :30-50%<br>P.aurelia :casual |

Table II.

| Paramoecium strain | dormant culture | date renewed cult.medium | conjugation in the renewed culture. |
|---|---|---|---|
| *17aIII* | Mar.15, 1919. | Mar.31 (aqueous) | Apr.10,about 30%<br>" 11, " 20% |
| *P.caudatum*, *17aIII*<br>(*P.aurelia*, *231* added on Mar.31) | Mar.15, 1919. | Mar.31 (aqueous) | *P.caudatum*:--<br>Apr.10,about 20%<br>" 11, " 30%<br>*P.aurelia*:--<br>Apr.12,one pair |
| *P.caudatum*, *17aIII*<br>(*P.aurelia*, *13a* added on Mar.31) | Mar.15, 1919. | Mar.31 (aqueous) | *P.caudatum*:--<br>Apr.10,casual<br>*P.aurelia*:--<br>none |

Table III.

(Each culture of the seven races listed in the first column was treated as here indicated.)

| | | | |
|---|---|---|---|
| 38b<br>39a<br>44a<br>57a<br>43a<br>2a<br>8a | Dormant since Nov.14 1919. Renewed on Mar.3, 1920. | Conjug.in 2a,44a and 57a (see p.16 of paper). | Experiments with each race as follows:--<br>Mar.9,+$AlCl_3$(.00003N)<br>" 9,+$FeCl_3$(.00002N)<br>" 12,+$AlCl_3$(.00003N)<br>" 12,+$AlCl_3$(.00009N)<br>" 12,+$NaNO_3$(.001N)<br>(Results essentially negative.See p.16of paper.) |

Table IV.

| dormant culture | date renewed; cult. medium | conjug. in renewed culture | sub-cultures 24 hrs. after treatment: conjugation |
|---|---|---|---|
| 13aII<br>Mar.28, 1919. | A. Apr. 11<br>(aqueous) | none | Apr. 14, untreated :none |
| | B. Apr. 23<br>(aqueous) | none | Apr. 25, untreated : "<br>May 3, +$NaNO_3$ (.001N) : 5%<br>" 4, untreated :casual<br>" 10, " :none<br>" 10, +$NaNO_3$ (.001N) : "<br>" 10, +$FeCl_3$ (.00002N) : " |
| | C. May 8<br>(aqueous) | May 13, casual<br>" 14, 3-5%<br>" 16, 10% | " 14, +$NaNO_3$ (.001N) : "<br>" 14, +$FeCl_3$ (.00002N) : " |
| | D. May 23<br>(aqueous) | May 30, casual | May 27, +$NaNO_3$ (.001N) :casual<br>" 27, +$FeCl_3$ (.00002N) :none |
| | E1. June 6<br>(aqueous) | none | June 10, untreated : "<br>" 10, +$NaNO_3$ (.001N) : "<br>" 10, +$FeCl_3$ (.00001N) : " |
| | E2. June 6<br>"Na+Ca sol."<br>*(=$NaNO_3$ .01N) | June 9, casual | " 10, untreated ::casual<br>" 10, +Na+Ca (.01N) : "<br>" 10, 10cc. culture+ 1cc. $FeCl_3$ (.0001N) 5%<br>" 10, 10cc. culture+ 2cc. $FeCl_3$ (.0001N) 20% |

Table V.

| dormant culture | date renewed cult. medium | conjug. in renewed culture | sub-culture 24 hrs. after treatment: conjugation |
|---|---|---|---|
| 14aII<br>Apr.11, 1919. | A. May 13 (aqueous) | none | May 17, +$NaNO_3$(.001N) :none |
| | | | " 31, " " : " |
| | B. May 26 (aqueous) | none | " 29, +$NaNO_3$(.001N) : " |
| | | | " 31, " " : " |
| | | | " 29, +$FeCl_3$(.00002N) : " |
| | | | " 31, " " : " |
| | C. June 14 "Na+Ca sol." (.01N) | June 19, casual | June 19, +Na+Ca(.01N) :casual |
| | | " 23, " | " 19, " (.03N) : " |
| | | | " 19, 2cc. culture+ 1cc. $FeCl_3$(.0002N) 5% |
| | | | " 21, +Na+Ca(.01N) :casual |
| | | | " 21, " (.03N) : 3% |
| | | | " 21, 1cc. culture+ 1cc. $FeCl_3$(.0002N) 3% |
| | D1. June 28 (aqueous) | none | |
| | D2. June 28 "Na+Ca sol." (.01N) | July 3, 1-3% | July 6, +Na+Ca(.01N) :none |
| | | " 6, casual | " 6, " (.015N) :casual |

Table VI.

| Paramoecium strain | dormant culture | date renewed cult.medium | conjugation in the renewed culture |
|---|---|---|---|
| 11j | July24, 1919. | A.Sept.19 (aqueous) | none |
| | | B.Sept.19 NaNO3(.002N) | Sept.26,casual |
| 11i | July24, 1919. | A.Sept.25 (aqueous) | none |
| | | B.Sept.25 NaNO3(.001N) | none |
| | | C.Sept.25 NaNO3(.002N) | Sept.30,one pair |
| | | D.Sept.25 NaNO3(.005N) | Sept.30,3-5 pairs |

Table VII.

| Paramoecium strain | dormant culture | date renewed; cult.medium | conjugation in the renewed culture |
|---|---|---|---|
| 1bI<br>(stock culture) | July24, 1919 | A.Sept.30<br>(aqueous) | Oct. 2, 5-10% |
| | | B.Sept.30<br>NaNO3(.0025N) | Oct. 2,casual<br>" 3, 5% |
| | | C.Sept.30<br>NaNO3(.005N) | Oct. 2,casual<br>" 3, 1-3% |
| | | D.Sept.30<br>Na+Ca(.005N) | Oct. 2,casual<br>" 3, " |
| 3d | July24, 1919, | A.Oct.4<br>(aqueous) | none |
| | | B.Oct.4<br>NaNO3(.001N) | Oct. 8, 1-3%<br>" 10, 1-5%<br>" 12,10-15% |
| | | C.Oct.4<br>NaNO3(.002N) | Oct. 8, 5-10%<br>" 13, 1-3% |

Table VIII.

| Paramoecium strain; duration of experiment. | aqueous medium | | | | | | Na+Ca med.( .005N ) | | | | | | NaNO3 med.( .005N ) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | lines | | | | | | lines | | | | | | lines | | | | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 |
| 1st.5da. | 9 | 9 | 9 | 9 | 9 | | 13 | 13 | 13 | 13 | 13 | | | | | | | |
| 11g: 2d. 5da. | 7 | 7 | 8 | 10 | 9 | | _ | 9 | 11 | 9 | _ | | | | | | | |
| 10da. | 16 | 16 | 17 | 19 | 18 | | _ | 22 | 24 | 22 | _ | | | | | | | |
| 1st.5da. | 6 | 7 | | | | | 11 | 10 | 10 | 11 | | | | | | | | |
| 38a: 2d. 5da. | 6 | 4 | | | | | 9 | 10 | 10 | 9 | | | | | | | | |
| 10da. | 12 | 11 | | | | | 20 | 20 | 20 | 20 | | | | | | | | |
| 1b: 1st.5da. | 4 | 7 | 5 | 7 | 7 | 5 | 10 | 12 | 11 | 11 | 12 | 11 | 11 | 13 | 13 | 10 | 10 | 13 |

(Division rate per line in three strains of Paramoecium growing in saline media, as compared with those in an aqueous medium. Hay infusion was used as the source of nutriment.)

# VITA

Hoyt S.Hopkins was born at Port Byron,Ill., on the 14th. of February,1893. He attended the high school at Oberlin,O.,and entered Oberlin College in 1910,taking the degree of Bachelor of Arts in 1914. The year 1914-'15 was devoted to advanced work in zoology at Oberlin,leading to the degree of Master of Arts in 1915. In the fall of 1915 he entered the Johns Hopkins University,serving as a student assistant to Dr.Caswell Grave for two years. Two summers were devoted to the study of marine biology at Woods Hole,Mass. He was appointed Adam T.Bruce Fellow in Zoology in 1917,and again in 1919,after having been absent from the university during the previous year while in government service. His subjects of study were Zoology, Chemistry and Animal Physiology.